Received: 21 April 2020 | Revised: 28 April 2020 | Accepted: 7 May 2020
DOI: 10.1111/pce.13787

Plant, Cell & Environment
WILEY

COMMENTARY

At the crossroads of strigolactones and abscisic acid pathways: A role for miR156

Guillaume Brun

Institute for Biology, Department for Systematic Botany and Biodiversity, Humboldt-Universität zu Berlin, Berlin, Germany

Correspondence
Guillaume Brun, Institute for Biology, Department for Systematic Botany and Biodiversity, Humboldt-Universität zu Berlin, D-10115 Berlin, Germany.
Email: guillaume.brun@hu-berlin.de, gbrun@uni-muenster.de

The changing climatic conditions have increased the occurrence of ecological disasters worldwide. Because it negatively affects plant growth and soil fertility, drought stress represents one of the most limiting environmental conditions for plant production. Drought stress is typically multi-dimensional. Its impacts on the plant depend on both the duration and the severity of water scarcity in the soil. This leads to many mitigation strategies at the plant morphological, physiological, and molecular level, all aiming at maintaining cell water homeostasis. One of the earliest responses to water deprivation is stomatal closure, which consequently decreases stomatal conductance and transpiration rate in order to stabilize the shoot water potential. Decades of research have repeatedly proven that the accumulation of the phytohormone abscisic acid (ABA) in guard cells drives stomatal closure (Hossain, Wani, Bhattacharjee, Burritt, & Tran, 2016).

More recently, strigolactones (SL) have emerged as other critical hormonal signals modulating multiple aspects of plant physiology, including systemic responses to abiotic stresses. Independent studies showed in *Arabidopsis thaliana*, *Solanum lycopersicum* (tomato), and *Lotus japonicus* (lotus) that SL-depleted mutants exhibited reduced stomatal closure and hypersensitivity to a diverse array of stresses (Bu et al., 2014; Liu et al., 2015; Van Ha et al., 2014; Visentin et al., 2016). Inversely, enhanced stomatal closure and overall amelioration of plant performance under drought were observed in plants exogenously supplied with SL (Lv et al., 2018; Visentin et al., 2016; Zhang, Lv, & Wang, 2018), and in plants for which SL production in the shoot was enhanced by grafting onto SL-depleted rootstocks (Visentin et al., 2016). What most of these studies consistently showed is that hypersensitivity to drought in SL-depleted lines correlated with lower ABA levels, and lower stomatal sensitivity to exogenous and/or endogenous ABA in stressed shoots (Bu et al., 2014; Liu et al., 2015; Lv et al., 2018; Van Ha et al., 2014; Visentin et al., 2016).

By means of grafting experiments, Visentin et al. (2016) proposed an elegant model in which the reduction of SL synthesis in water-deprived tomato roots is a systemic stress signal causing SL accumulation in the shoots and a higher sensitivity to ABA in guard cells. The authors built on this research and investigated how ABA and SL pathways inter-connect with respect to stomatal regulation upon drought stress in tomato. In this issue of *Plant, Cell & Environment*, Visentin et al. (2020) provide pioneering evidence that one particular microRNA, miR156, is a critical downstream effector contributing to SL-induced drought tolerance through an ABA-dependent pathway.

1 | STRIGOLACTONES AND ABA PATHWAYS CROSSTALK THROUGH MIR156

MicroRNAs are a class of 19-24 nucleotides-long non-coding RNA that modulates gene expression at the transcriptional, post-transcriptional and translational level. Many miRNAs display species-specific expression patterns and targets, while others happen to be highly conserved. In this line, miR156 consistently accumulates upon a variety of abiotic stresses in multiple species, and miR156 over-expressing genotypes (hereafter referred to as "miR156-oe") outperform their wild-type counterparts in *Arabidopsis* and *Medicago sativa* under adverse conditions (Arshad, Feyissa, Amyot, Aung, & Hannoufa, 2017; Cui, Shan, Shi, Gao, & Lin, 2014).

In this issue, Visentin et al. determined that a tomato miR156-oe line displayed a lower stomatal conductance than the wild-type in optimal conditions, yet with similar leaf water potential. Although both genotypes responded to drought by a progressive decrease in stomatal conductance, the drop in leaf water potential was markedly lower in miR156-oe lines. Interestingly, leaf water potential re-increased up to normal levels in both lines after rehydration, while stomatal conductance failed to recover in the miR156-oe line. Therefore, miR156 accumulation would both limit water loss through transpiration during drought and delay stomatal re-opening upon

rehydration, thereby slowing plant responses to abrupt changes in water availability. Somewhat counter-intuitively, such a better acclimation to drought was associated with less endogenous ABA in miR156-oe leaves, which correlated with a stronger expression of *CYP707A* genes encoding ABA catabolic enzymes. Moreover, the miR156-oe line exhibited higher sensitivity to ABA since its stomatal conductance and aperture decreased much more and at lower concentrations upon exogenous application of ABA.

Because these characteristics perfectly matched those of SL-depleted lines (Visentin et al., 2016), the authors hypothesized a causality between SL and miR156 accumulation for stomatal functioning during drought response. Accordingly, Visentin et al. (2020) observed that an exogenous application of SL on unstressed leaves of the wild-type mimicked the effect of drought on miR156 accumulation, while the SL-depleted line failed to accumulate miR156 transcripts. As such, stomatal conductance recovered to normal levels after rehydration at a faster rate in SL-depleted plants than in the wild-type. Exogenous supply with SL prior to water deprivation-induced primed responses, leading to higher miR156 levels compared to the mock-treated control under drought conditions, which kept increasing substantially after rehydration. Associated with this was a failure of stomatal conductance to recover to normal levels after rehydration, thereby phenocopying the miR156-oe line.

Taken together, these results indicate that drought-induced SL accumulation in the leaves triggers the synthesis of miR156, which in turn increases guard cells sensitivity to ABA and results in stomatal closure (Figure 1). In this framework, miR156 would mainly operate after drought, especially by improving plant performances upon rehydration through a delayed stomatal reopening. In line with this hypothesis, the authors observed that several known targets of miR156, especially members of the SPL (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE) family, were strongly up-regulated in SL-depleted lines after drought.

2 | DROUGHT STRESS: A MODEL SYSTEM TO UNRAVEL MOLECULAR INTERACTIONS BETWEEN STRIGOLACTONES AND ABA METABOLISM AND SIGNALING PATHWAYS

Both ABA and SL derive from a common carotenoid precursor, which initially led to the hypothesis that their biosynthesis pathways might

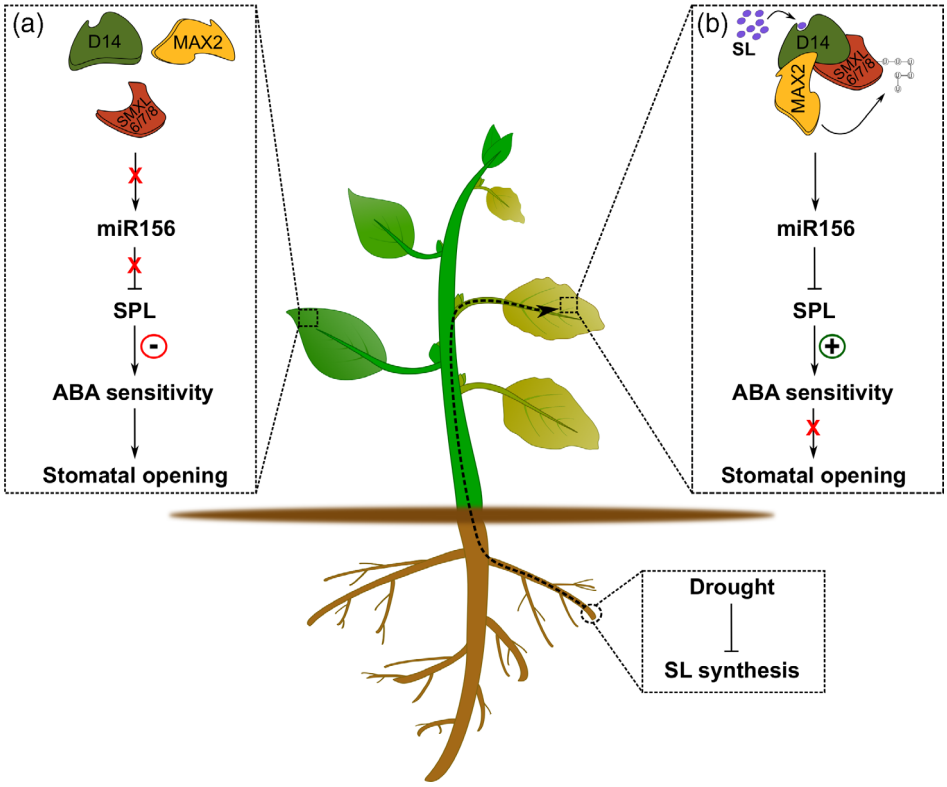

FIGURE 1 Hypothetical model for strigolactone (SL)-induced drought tolerance through ABA-dependent pathway. (a) Well-watered plants possess low shoot SL levels. The presence of functional SMXL6,7,8 transcriptional repressors inhibits miR156 biogenesis. In turn, SPL (squamosa promoter binding protein-like) proteins accumulate, which contribute to lowering ABA sensitivity in guard cells and maintaining opened stomata. (b) Inhibition of SL biosynthesis in the roots upon water deprivation acts as a signal to stimulate SL biosynthesis in the leaves (SL, blue circles). Strigolactone perception by D14 enables the interaction with the F-box protein MAX2, which supposedly ubiquitinates SMXL6,7,8 (suppressor of max2-1 like), thereby lifting downstream transcriptional inhibition. Consequently, miR156 transcripts accumulate and inhibit transcription of *SPL* genes. This contributes to increase sensitivity to ABA in guard cells, and to maintain stomatal closure during and after drought stress. Adapted from Brun et al. (2018)

be inter-regulated. A first empirical evidence was that tomato ABA-deficient mutants, or wild-type plants treated with specific inhibitors of the ABA biosynthetic enzymes NCED (9-cis epoxycarotenoid dioxygenase), displayed reduced SL levels in roots (López-Ráez et al., 2010). Inversely, chemical inhibition of SL biosynthetic enzymes CCD7 and CCD8 (Carotenoid Cleavage Dioxygenase) did not alter root ABA levels (López-Ráez et al., 2010), altogether suggesting that ABA influences SL metabolism but not vice versa.

It is only by studying shoot responses to abiotic stresses that researchers first demonstrated ABA synthesis modulation by SL. Indeed, shoots of CCD7-silenced lotus plants displayed significantly lower ABA levels than the wild-type upon a combined osmotic stress and phosphate deprivation, while no changes were observed in unstressed plants or plants subjected to either of these stresses (Liu et al., 2015). Similarly, in tomato, drought induced a much more severe decrease in shoot ABA contents in CCD7-silenced plants than in the wild-type, whereas only slight variations occurred in unstressed leaves (Visentin et al., 2016). Interestingly, the interaction between SL and ABA synthesis pathways might display opposite patterns in monocots, since rice mutants devoid of CCD7 or CCD8 encoding genes contained higher shoot ABA levels in optimal conditions, and even higher levels upon water deprivation. As a result, SL-depleted rice mutants exhibited a better tolerance to drought (Haider et al., 2018).

How SLs influence root ABA contents under stress is arguably less documented, although similar synergistic patterns are observed in tomato and lotus (Liu et al., 2015; Visentin et al., 2016). It is however important to note that pre-treatment of lotus roots with the synthetic SL analogue GR24 prior to osmotic stress did not result in increased root ABA levels (Liu et al., 2015), suggesting that SL might, in some instances, negatively affect ABA metabolism as well. Altogether, these studies illustrate that the direction, the type, and the amplitude of the interactions between SL and ABA pathways strongly depend on a vast number of parameters (i.e., duration/severity/type of stress, species, developmental stage, territory, etc.). Most importantly, however, they especially reflect that SL-ABA interactions amplify upon stress responses, making the study of drought a timely and powerful system to investigate molecular actors involved in this hormonal crosstalk. In this respect, the study by Visentin et al. provides the first empirical evidence that miRNAs are key elements acting downstream of SL signaling to regulate ABA metabolism and sensitivity.

3 | LOOKING BEYOND STRIGOLACTONES SYNTHESIS AND PERCEPTION

Strigolactones have been known since the 1960s for their ability to stimulate seed germination of parasitic weeds when exuded by the roots into the rhizosphere (Brun et al., 2018). Nonetheless, increasing interest into SL biology only came in the late 2000s with the discovery that these molecules also act as hormones *in planta*, with pervasive roles throughout plant development (Waters, Gutjahr, Bennett, & Nelson, 2017). With more than 600 publications, the past decade has seen remarkable progress especially in identifying molecular components involved in SL biosynthesis and perception. The story presented here constitutes a key complement to ongoing international research lines, and paves the way for a thorough description of the molecular events at play between SL perception and transcriptional responses.

There are still numerous missing pieces in the proposed framework. For example, there is little empirical evidences that SLs accumulate in the shoots upon drought stress, since the SL quantity in above-ground plant parts still falls below the detection threshold. Previous results along with the present study by Visentin et al. (2020) suggest that the accumulation of SL in the shoots is directly responsible for miR156 synthesis (Figure 1). Yet, the mechanisms underlying SL accumulation in the shoot upon low root SL levels are unresolved, and the hypothesis that low SL contents belowground is the direct triggering signal for miR156 production aboveground needs to be addressed through, for example, reciprocal grafts.

In relation to this, the signaling pathway between SL perception and miR156 synthesis remains elusive. Strigolactone perception by the α/β hydrolase D14 (DWARF14) induces the recruitment of the the F-box protein MAX2 (MORE AXILLARY GROWTH2), which supposedly triggers downstream ubiquitination of transcriptional repressors of the SMXL (SUPPRESSOR OF MAX2-LIKE) family (Waters et al., 2017). Among the eight SMXL paralogs encoded in *Arabidopsis*, SMXL6, 7, and 8 redundantly repress SL signaling, which affects shoot and root architecture and leaf shape (Soundappan et al., 2015; Wang et al., 2015). A very recent paper demonstrated that the *Arabidopsis smxl6,7,8* triple mutant is more tolerant to drought than the wild-type, which correlates with increased ABA sensitivity in cotyledon opening (Li et al., 2020). These results are in accordance with the study by Visentin et al., since mutation of SMXL6,7,8 would lift the repression of SL signaling and therefore mimic an over-production of SL. Such consistency between studies makes it worth investigating the role of SMXL6,7,8 and putative associated partners in the regulation of miR156 biogenesis upon drought-induced SL accumulation (Figure 1).

Finally, Visentin et al. (2020) propose that the effects of SL-induced miR156 regulation on ABA metabolism and stress recovery require members of the SPL family. While *SPL* transcripts are known targets of miR156, their ultimate function in that framework is still unknown. The accessibility to gain- and loss-of-function mutants with strong phenotypes under stress is therefore an asset for comparative transcriptomics under stress induction and recovery.

These few sample questions illustrate the current gaps in our understanding of molecular components downstream of SL signaling, and their interaction with other hormonal pathways in plant development. However, it is urgently needed in order to address upcoming food security issues. In relation to this, rice, one of the most important crop worldwide, shows opposite responses to the dicot species studied so far. The use of multiple species to address the current lack of knowledge is therefore not only relevant for applied research, but also for understanding the evolutionary trajectories of SL involvement in stress responses.

CONFLICT OF INTEREST

The author has declared no conflict of interest.

ORCID

Guillaume Brun https://orcid.org/0000-0001-5551-0604

REFERENCES

Arshad, M., Feyissa, B., Amyot, L., Aung, B., & Hannoufa, A. (2017). MicroRNA156 improves drought stress tolerance in alfalfa (*Medicago sativa*) by silencing *SPL13*. *Plant Science*, *258*, 122–136.

Brun, G., Braem, L., Thoiron, S., Gevaert, K., Goormachtig, S., & Delavault, P. (2018). Seed germination in parasitic plants: What insights can we expect from strigolactone research? *Journal of Experimental Botany*, *69*(9), 2265–2280.

Bu, Q., Lv, T., Shen, H., Luong, P., Wang, J., Wang, Z., … Huq, E. (2014). Regulation of drought tolerance by the F-box protein MAX2 in arabidopsis. *Plant Physiology*, *164*(1), 424–439.

Cui, L. G., Shan, J. X., Shi, M., Gao, J. P., & Lin, H. X. (2014). The miR156-SPL9-DFR pathway coordinates the relationship between development and abiotic stress tolerance in plants. *Plant Journal*, *80*(6), 1108–1117.

Haider, I., Andreo-Jimenez, B., Bruno, M., Bimbo, A., Flokova, K., Abuauf, H., … Ruyter-Spira, C. (2018). The interaction of strigolactones with abscisic acid during the drought response in rice. *Journal of Experimental Botany*, *69*(9), 2403–2414.

Hossain, M. A., Wani, S. H., Bhattacharjee, S., Burritt, D. J., & Tran, P. (2016). *Drought stress tolerance in plants* (Vol. 1). Switzerland: Springer.

Li, W., Nguyen, K. H., Tran, C. G., Watanabe, Y., Tian, C., Yin, X., … Yamaguchi, S. (2020). Negative roles of Strigolactone-related SMXL6,7 and 8 proteins in drought resistance in *Arabidopsis*. *Biomolecules*, *10*, 607.

Liu, J., He, H., Vitali, M., Visentin, I., Charnikhova, T., Haider, I., … Cardinale, F. (2015). Osmotic stress represses strigolactone biosynthesis in *Lotus japonicus* roots: Exploring the interaction between strigolactones and ABA under abiotic stress. *Planta*, *241*(6), 1435–1451.

López-Ráez, J. A., Kohlen, W., Charnikhova, T., Mulder, P., Undas, A. K., Sergeant, M. J., … Bouwmeester, H. J. (2010). Does abscisic acid affect strigolactone biosynthesis? *New Phytologist*, *187*(2), 343–354.

Lv, S., Zhang, Y., Li, C., Liu, Z., Yang, N., Pan, L., … Wang, G. (2018). Strigolactone-triggered stomatal closure requires hydrogen peroxide synthesis and nitric oxide production in an abscisic acid-independent manner. *New Phytologist*, *217*(1), 290–304.

Soundappan, I., Bennett, T., Morffy, N., Liang, Y., Stanga, J. P., Abbas, A., … Nelson, D. C. (2015). SMAX1-LIKE/D53 family members enable distinct MAX2-dependent responses to strigolactones and karrikins in *Arabidopsis*. *Plant Cell*, *27*, 3143–3159.

Van Ha, C., Leyva-Gonzalez, M. A., Osakabe, Y., Tran, U. T., Nishiyama, R., Watanabe, Y., … Yamaguchi-Shinozaki, K. (2014). Positive regulatory role of strigolactone in plant responses to drought and salt stress. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(2), 851–856.

Visentin, I., Pagliarani, C., Deva, E., Caracci, A., Tureckova, V., Novak, O., … Cardinale, F. (2020). A novel strigolactone-miR156 module controls stomatal behavior during drought recovery. *Plant, Cell and Environ.*, *43*, 1613–1624.

Visentin, I., Vitali, M., Ferrero, M., Zhang, Y., Ruyter-Spira, C., Novak, O., … Cardinale, F. (2016). Low levels of strigolactones in roots as a component of the systemic signal of drought stress in tomato. *New Phytologist*, *212*(4), 954–963.

Wang, L., Wang, B., Jiang, L., Liu, X., Li, X., Lu, Z., … Li, J. (2015). Strigolactone signaling in *Arabidopsis* regulates shoot development by targeting D53-like SMXL repressor proteins for ubiquitination and degradation. *Plant Cell*, *27*, 3128–3142.

Waters, M. T., Gutjahr, C., Bennett, T., & Nelson, D. C. (2017). Strigolactone signaling and evolution. *Annual Review of Plant Biology*, *68*, 291–322.

Zhang, Y., Lv, S., & Wang, G. (2018). Strigolactones are common regulators in induction of stomatal closure in planta. *Plant Signaling & Behavior*, *13*(3), e1444322.

How to cite this article: Brun G. At the crossroads of strigolactones and abscisic acid pathways: A role for miR156. *Plant Cell Environ*. 2020;43:1609–1612. https://doi.org/10.1111/pce.13787